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CHIMERIC ANALYSIS OF HEMOPOIETIC CELLS AFTER CROSS-SEX PARABIOSIS

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Abstract: Anemia or polycythemia was induced in male rats. These males were conjugated with healthy, untreated female litter mates by FANG's method of aortic parabiosis that resulted in complete cross circulation of blood between the two animals. The sex chromosomes of cells in erythropoiesis in various hemopoietic organs were examined in the treated male animals. The anemic parabionts indicated sharp increases in chimerical rates with erythroid marrows being evident. Polycythemic parabionts indicated marked decreases in chimerical rates with evidence of myeloid marrows. These findings suggested that the so-called stem cells in peripheral blood of the female parabiont migrated to the bone marrow of the male partner and that these migrating cells differentiated to erythroblast. The possible relationships between erythropoiesis and other cell proliferations in the hemopoietic organs are discussed.

Investigations of radiation chimera have revealed that fresh bone marrow cells introduced into lethally irradiated animals participate in hemopoiesis in the bone marrow and spleen (1-8). Recent parabiotic investigations of irradiated rats conjugated with untreated rats have shown that circulating nucleated cells of the untreated partner were involved in lymphopoiesis in the lymph nodes and thymus, and in hemopoiesis in the bone marrow and spleen (9, 10). Other parabiotic studies have demonstrated that the rate of bone marrow chimerism is lower than that of the lymph nodes (11, 12). These findings have suggested some differences in chimeric rates between lymphoid, myeloid and erythroid cells of the bone marrow.

In the present study the author has examine sex chimerism during erythropoiesis in the hemopoietic organs of anemic and polycythemic male rats that were conjugated by parabiosis with healthy female litter mates. The cells involved in erythropoiesis in the male bone marrow were mostly cells of the female partner.

MATERIALS AND METHODS

Seventy-eight inbred Wistar rats, 69 males and 9 females, weighing 230-300g were used. The animals were assigned to three groups: 13 to the anemia

group, 44 to the polycythemia group and 21 to the healthy untreated group. The 13 animals of the anemia group were further divided: 5 male controls, 2 anemia males conjugated with 2 healthy females and 4 male aorta donors for transplantation in parabiosis. The 44 animals of the polycythemia group were divided into four subgroups: 4 male controls, 3 polycythemia males conjugated with 3 healthy females, 28 male blood donors and 6 male aorta donors. The 21 rats of the healthy, untreated group were divided into three subgroups: 5 male controls, 4 healthy males conjugated with 4 healthy females and 8 male aorta donors.

Parabiosis was performed by the FANG method of aortic anastomoses allowing for cross circulation of blood between the two animals (13, 14). All male animals joined in parabiosis were conjugated with healthy female litter mates. Anemia was induced by bloodletting of 2-3 ml from the orbital sinuses once daily for 4 consecutive days and by bloobletting of 4-5 ml from the left renal vein on the final day prior to parabiosis.

The definition of anemia was a composite of: (a) hematocrit (Ht) level lower than 25%, (b) red blood cell (RBC) count lower than 500×10^4 per cmm and (c) reticulocyte (RC) percentage in peripheral blood larger than 10%. Polycythemia was induced by transfusion of RBC from an animal of the same strain. RBC were suspended in physiological saline, 14×10^4-16×10^4 cells per cmm, and injected at a total dose of 5 ml per animal per injection day (4 days within 6 consecutive days) into the dorsal vein of the penis and abdominal cavity. The definition of polycythemia was a composite of: (a) Ht value higher than 60%, (b) RBC count larger than 900×10^4 per cmm and (c) RC percentage lower than 0.5%. The anemic and polycythemic controls were sacrificed one day after the final bloodletting or transfusion; the healthy controls were sacrificed on the same day.

In the experimental groups parabiosis was performed one day after the final treatment for anemia or polycythemia. Blood loss due to the parabiotic operation was less than 1.0 ml. Penicillin was administered at a dose of 10,000 U per day after parabiosis to prevent postoperative infection. The parabionts were sacrificed under light ether anesthesia by dissecting the transplanted aortas 120 hours after parabiosis. At 3 hours prior to sacrifice, colchicine was injected subcutaneously at 1.0 mg per Kg. Blood was collected just prior to sacrifice from the orbital sinus for Ht value, RBC count, RC percentage and for smear specimens. RC percentage was determined under supravital staining with brilliant cresyl blue and on smear specimens prepared with Giemsa staining. Smear specimens for morphological examination were stained with May-Grünwald-Giemsa. After sacrifice, the femoral bone marrow, spleen, lymph nodes (neck and mesenteric) and thymus were prepared for morphologic observation and for chromosome analyses. The tissue smears and imprints were fixed and stained with May-Grünwald-Giemsa. Tissue sections were fixed in 10% formol, embedded in paraffin and stained with Hematoxylin-Eosin.

For chromosome analysis the tissues were rinsed in physiological saline and cut into small pieces by blunt dissection. The cell suspensions obtained

were filtered through a nylon mesh to remove the gross tissue debris and the cells were sedimented from the medium by centrifugation at 1,500 rpm for 5 min.. The sedimented cells were examined by the OMURA (15) method for chromosome analysis. Microphotographs of 67 to 260 chromosome sets were analysed for each organ.

RESULTS

At 5-days after parabiosis of healthy-to-healthy animals, the Ht value, RBC count and RC percentage showed no marked changes but slight anemia was present compared to the period prior to parabiosis (Table 1).

TABLE 1 MEAN HEMATOCRIT (Ht) VALUE, RED BLOOD CELL (RBC) COUNT AND RETICULOCYTE (RC) PERCENTAGE IN PERIPHERAL BLOOD OF RATS.

Condition	Number pairs	Before parabiosis			5-Days after parabiosis *		
		Ht (%)	R B C ($\times 10^4$/cmm)	R C (%)	Ht (%)	R B C ($\times 10^4$/cmm)	R C (%)
Healthy	4	47.5	756	2.1	41.7	724	2.7
Anemia	2	24.5	475	11.2	29.8	499	11.0
Polycythemia	3	62.5	941	0.2	50.3	770	0.9

* Parabiosis with a healthy, untreated female litter mate.

The bloodletting process produced severe anemia. One day after the final bleeding, the analysis indicated Ht levels at 23–26%, RBC at 450×10^4–500×10^4 per cmm and RC percentages of 10–13%. In one parabiotic pair of the anemic group, the Ht value, RBC count and RC percentage failed to recover by 5-days after parabiosis but these indices increased in the other parabiotic pair.

Under RBC transfusions, remarkable polycythemia were induced: Ht levels at 60–63.5%, RBC counts at 930×10^4–950×10^4 per cmm and RC percentages of 0–0.3%. Five-days after parabiosis these values decreased moderately but polycythemia was still evident: Ht levels at 50–56%, RBC counts at 670×10^4–870×10^4 and RC percentages of 0.5–1.8%.

White blood cell (WBC) classification showed some specific changes under both anemic and polycythemic conditions before parabiosis (Table 2). Lymphocytosis was induced by anemia with a decrease of granulocytes while under polycythemia granulocytes increased. In anemia animals 5-days after parabiosis, lymphocyte and granulocyte activity appeared normal. In polycythemia animals 5-days after parabiosis, granulopenia resulted with some lymphocytosis.

In tissue sections of healthy-to-healthy parabionts, no remarkable changes were observed in the bone marrows (Fig. 1-a) and no extramedullary hemopoiesis in the spleen was observed. In some cases slight lymphocyte depletion

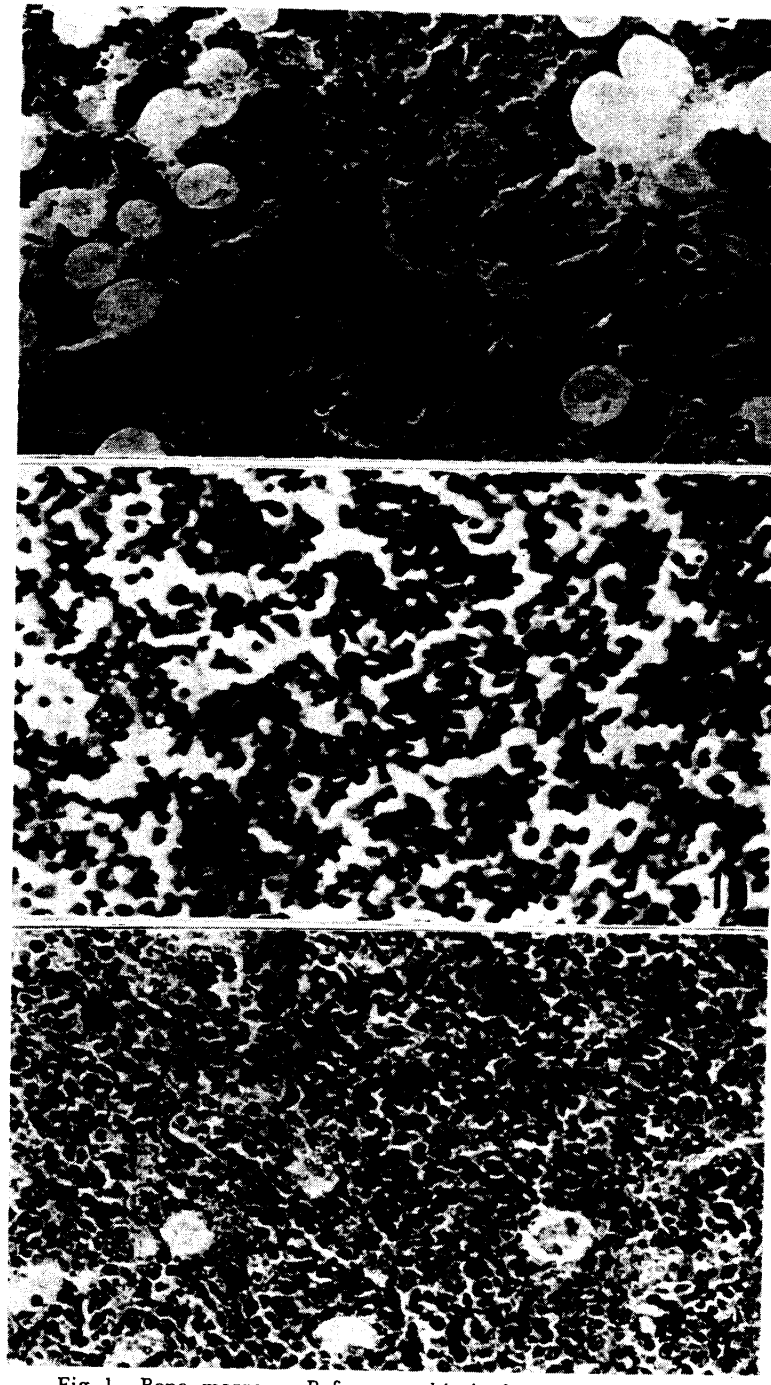

Fig. 1. Bone marrow. Before parabiosis from a healthy rat (1-a), anemia-induced erythroid hyperplasia (1-b) and polycythemia-induced myeloid marrow (1-c).

TABLE 2 MEAN WHITE BLOOD CELL CLASSIFICATION (%) IN PERIPHERAL BLOOD OF RATS.

Condition	Number pairs	Before parabiosis					5-Days after parabiosis				
		Ly	Seg	Metamy	My	Mo	Ly	Seg	Metamy	My	Mo
Healthy	4	69.5	23.6	1.5	0	5.5	62.0	29.5	1.2	0.8	6.5
Anemia	2	81.0	10.0	0.7	0	8.3	62.5	27.5	2.0	0.5	7.5
Polycythemia	3	56.3	34.6	0.7	0	8.4	73.3	16.5	1.3	0	8.9

Ly, lymphocytes; Seg, segmented granulocytes; Metamy, metamyelocytes; My, myelocytes; Mo, monocytes.

were observed in the spleen, lymph nodes and thymus. In anemic animals before parabiosis a marked erythroid hyperplasia was observed in the bone marrow (Fig. 1-b), extra-medullary hemopoiesis in the spleen and moderate lymphocyte depletions in the spleen, lymph nodes and thymus. Five-days after parabiosis marked erythroid hyperplasia was still present in the bone marrow with extra-medullary hemopoiesis in the spleen and moderate lymphocyte depletions in the spleen, lymph nodes and thymus.

In polycythemic animals before parabiosis, bone marrow erythropoiesis was strikingly suppressed showing evidence of myeloid marrow with a number of undifferentiated cells (Fig. 1-c). The spleen had no erythropoietic focus, and moderate lymphocyte depletions were observed in the spleen, lymph nodes and thymus. Five-days after parabiosis the bone marrow still showed marked erythroid hypoplasia as in the polycythemic controls. Moderate lymphocyte depletions were still seen in the spleen, lymph nodes and thymus.

Myelogram examination before parabiosis indicated that the percentage of erythroblasts in the entire nucleated cell population was 29% in healthy controls, 51% in anemic animals and 4% in polycythemic animals (Table 3). In anemic animals before parabiosis, the percentages of myeloid cells and especially lymphoid cells were low. In polycythemic animals before parabiosis lymphocytes showed high values and erythroblasts indicated low values. Five-days after parabiosis erythroblasts decreased slightly in anemic animals and increased in polycythemic animals but no remarkable changes were present in control parabionts. Lymphocytes showed no obvious changes in anemic animals but in polycythemic animals lymphocytes decreased. Myeloid cells increased in normal and anemic animals but showed no marked changes in polycythemic animals before and after parabiosis.

In healthy-to-healthy parabionts, the mean bone marrow mitotic index was 20‰ and increased to 23‰ 5 days after parabiosis (Table 4). In anemic animals before parabiosis the mean bone marrrow mitotic index was 115‰ and remained at this range after parabiosis. In polycythemic animals before parabiosis the mean bone marrow mitotic index was about 1.5‰ but increased

TABLE 3 MYELOGRAM (%) OF MALE RATS.

Cell type	Before parabiosis			5-Days after parabiosis		
	Healthy	Anemia	Polycythemia	Healthy	Anemia	Polycythemia
Myeloid series	37.7	27.4	53.3	51.1	47.9	53.9
Myeloblast	2.3	4.3	4.3	3.2	6.8	11.3*
Promyelocyte	3.0	8.2	3.2	6.3	18.6	19.1
Myelocyte	4.3	4.1	6.7	5.3	6.5	4.7
Metamyelocyte	6.7	1.8	13.0	7.9	6.6	6.0
Segmented neutrophile	17.1	4.0	18.8	22.9	4.4	6.3
Eosinophile	4.3	5.0	7.3	5.5	5.0	6.5
Erythroid series	29.1	50.8	3.7	28.2	37.4	15.0
Proerythroblast	0.3	3.3	0	0.9	3.9	0.3
Basophilic erythroblast	1.8	4.0	0.3	3.2	4.6	0.8
Polychromatic erythroblast	5.5	22.3	0.7	8.2	16.9	5.0
Orthochromatic erythroblast	21.5	21.2	2.7	15.9	12.0	8.9
Lymphoid series	28.1	9.9	38.6	16.6	8.4	25.2
Mature lymphocyte	26.2	8.4	36.1	16.0	7.4	24.5
Immature lymphocyte	1.5	1.5	2.5	0.6	1.5	0.7
Others	5.1	8.3	5.0	4.9	5.7	6.8
Monocyte	2.6	5.8	3.1	2.8	3.8	2.5
Plasma cell	1.5	2.2	1.3	1.8	1.9	2.0
Reticulum cell	1.0	0.3	0.6	0.5	0	2.3

Value represent means. * Includes undifferentiated cells.

TABLE 4 MEAN MITOTIC INDICES (‰) OF HEMOPOIETIC ORGANS OF MALE RATS.

Condition	Number pairs	Before parabiosis				5-Days after parabiosis			
		Bone marrow	Spleen	Lymph node	Thymus	Bone marrow	Spleen	Lymph node	Thymus
Healthy	4	20.0	1.0	5.0	2.5	23.0	10.7	10.7	9.7
Anemia	2	115.0	21.3	7.6	6.0	102.5	46.0	9.5	11.0
Polycythemia	3	1.5	1.0	1.5	0	16.0	10.3	8.3	12.7

Values represent mitotic cells per 1,000 nucleated cells.

to 16‰ 5-days after parabiosis. The bone marrow and spleen of the anemic group showed consistently high mitotic indices.

The sex chromosome analysis of hemopoietic organs is shown in Table 5. In the bone marrow of the polycythemic group 5-days after parabiosis

TABLE 5 NUMBER OF MITOTIC CELLS EXAMINED IN CHROMOSOME ANALYSES OF HEMOPOIETIC ORGANS AND THE PERCENTAGE OF FEMALE CELLS IN MALE PARABIONTS.

Parabionts	Number pairs	Bone marrow				Spleen				Lymph node				Thymus			
		Male	Female	Total	%*	Male	Female	Total	%	Male	Female	Total	%	Male	Female	Total	%
Healthy-to-healthy	4	163	23	186	12.4	94	41	135	30.4	61	34	95	35.8	108	30	138	21.7
Anemic-to-normal	2	200	60	260	23.1	48	26	74	35.1	56	47	103	45.6	43	24	67	35.8
Polycythemic-to-normal	3	204	9	213	4.2	45	16	61	26.2	73	29	102	28.4	82	14	96	14.6

* Chimeric percentage (Female/Total).

a very low chimeric rate (4.2%) was found, while in the anemic group a high chimeric rate was present in the lymph nodes (45.6%). The chimeric percentages of the organs decreased in the order of lymph nodes, spleen, thymus and bone marrow.

DISCUSSION

The present investigation revealed that bone marrow chimeric rates after parabiosis were largely affected by the intensity of erythropoiesis. Very high chimeric rates were found in anemic animals and low rates in polycythemic animals.

In anemic animals the mitotic indices showed high values in bone marrow cells of the erythroid marrow and in the spleen with extra-medullary hemopoiesis, and most cells undergoing mitosis were erythroblasts. In contrast, mitotic indices were very low in polycythemic animals where erythroblasts were rarely encountered on smear specimens.

These results clearly indicate that the high chimeric rates in the bone marrow and spleen of anemic animals were due to the migration of erythroid precursor cells from the healthy partner through the blood stream. The migrating cells participated in erythropoiesis in the erythroid marrow and spleen with extra-medullary hemopoiesis. The low chimeric rate in the bone marrow of polycythemic animals, indicating suppression of erythropoiesis, is consistent with the data of the anemic animals.

In the present study, erythroid cells undergoing mitosis were not encountered in polycythemic animals and most cells in mitosis were granulocytes. However, it must be noted that mitotic indices of bone marrow cells were extremely low in polycythemic rats. The high chimeric rates of erythroid marrow under the anemic condition and the low chimeric rates of myeloid marrow under the polycythemic condition do not necessarily signify

the existence of low chimeric rates in other cell types aside from erythroid cells.

In lymph nodes, thymus and spleen the chimeric rates appeared somewhat lower in polycythemic animals but the rates were higher compared to those of the polycythemic bone marrow. It is uncertain why the bone marrow shows very low chimeric rates compared to other organs. As erythroid cells indicate a marked chimera in the bone marrow, the myeloid precursor cells or the bone marrow itself may have resistance to the acceptance of migrating cells of the partner, because myelopoiesis in specific cell strains may be specific to the bone marrow.

Lymphoid cells indicate high chimeric activity as seen in the data of lymph nodes, thymus and spleen. The bone marrow had a large number of migrating cells but these cells seemed not to be involved in chimerism, or their mitoses may have been extremely suppressed. Erythroid precursor cells (stem cells) could not be detected in spite of careful examination for immature erythroblasts in smear specimens of circulating blood after parabiosis, but the morphology of these circulating stem cell is uncertain. It appears reasonable, however, based upon the findings of erythropoiesis that stem cells from the circulating blood migrated to the bone marrow and differentiated to erythroblast.

It is of interest to note that the relative population of the bone marrow lymphoid cells was extremely low under anemia and high under polycythemia. Myeloid cells in the bone marrow indicate normal distribution after parabiosis in both anemic and polycythemic animals, but younger precursors and promyelocytes increased. The younger precursor cells and promyelocytes may be influencing the bone marrow chimeric rates, but further observations are required on granulopoiesis and myeloid hyperplasia for definitive conclusions.

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